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(71) Applicant *(for all designated States except US)*: NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: CHRISTENSEN, Flemming, M. [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK).

(74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).

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(54) **Title:** ORAL CARE COMPOSITIONS

(57) **Abstract**

The present invention relates to oral care compositions and products, such as dentifrices. According to the invention oral care compositions and products may advantageously comprise a protease, which is substantially inactive in the natural environment prevailing in the mouth, when the oral care composition or product is absent. Further the invention relates to the use of proteases, substantially inactive in the natural environment prevailing in the mouth, in *e.g.* oral care compositions or products. A final object of the invention is to provide a method for using said oral care products.

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Title: Oral care compositions

FIELD OF THE INVENTION

The present invention relates to oral care compositions and products comprising certain proteases.

Also contemplated is the use of said proteases for *e.g.* oral care purposes, and a method for using oral care products of the invention.

BACKGROUND OF THE INVENTION

One of the major events for newly hatched parents is the appearance of the first tooth in the mouth of their baby. From that day on the caring parents include dental care as a natural part of taking care of their baby.

To be able to sufficiently guarantee the capability of chewing, *e.g.* foods, during a whole lifetime it is necessary to keep the teeth in a good condition and to obtain a good oral hygiene.

To obtain and maintain a good dental and oral hygiene the teeth must at least be brushed once every day using toothpaste or the like. Further the mouth should regularly be rinsed with a mouth wash.

The brushing of the teeth primarily helps removing food particles from the teeth, and constitutes an important first step in preventing dental caries, which may cause dental holes, and further in preventing dental diseases.

A good dental hygiene should also be obtained to prevent formation of plaque and tartar, outbreak of oral diseases, and adherence of stains from tea, coffee and tobacco smoking, and

the like, which will remove the basis for obtaining a good appearance when *e.g.* smiling. Furthermore, a number of unpleasant incidences can be avoided by using oral care products for obtaining and/or maintaining *e.g.* a fresh breath, as most people dislike a in general bad breath, or *e.g.* an occasional garlic smelling breath.

Dental caries, plaque and tartar

In general it is believed that dental caries arise when cariogenic microorganisms, such as *Streptococcus mutans* or *Streptococcus sanguis*, grow in oral cavities. Sucrose, derived from foods, can be converted into water-soluble and insoluble polysaccharides by glucosyltransferase (GTF) produced by *e.g* the above mentioned cariogenic microorganisms. These polysaccharides coat the surface of the cariogenic microorganisms and other bacteria, and finally adhere onto the tooth surface to form a dental plaque. Bacteria contained in the dental plaque can degrade the polysaccharides to acids, such as lactic acid, which can lyse the tooth enamel, and may result in dental holes. As plaque continues to accumulate rock hard white or yellowish deposits may arise. These deposits are called calcified plaque, calculus or tartar, and are formed in the saliva from plaque and minerals, such as in particular calcium. Accumulation of tartar below the gum line may cause periodontal disease.

To prevent the formation of dental caries, plaque, and tartar, oral care compositions and products comprising enzymes, including proteases, glucose oxidase, amylases, lipase, peroxidases, have been suggested.

The use of proteolytic enzymes are today successfully used in connection with cleaning of dentures, and have also been suggested for oral care products *e.g.* for removing food remnants containing proteins, and for dissolving the proteinaceous matrix of tartar, which is attached to the dentin

and enamel. However, the use in oral care products has not been successful as said use is connected with serious disadvantages. First of all proteases have a damaging impact on the gums (and teeth) in the mouth when being exposed for a period of time.

Further, most proteases are inhibited by compounds, such as H_2O_2, which can be used advantageously in oral care products.

Examples of suggested proteolytic enzymes for oral care compositions and products include carboxypeptidase, pepsin, trypsin, pancreatic chymotrypsin, cathepsin, papain, and certain fungal derived proteases (see e.g. DE patent 734,765 and GB patent 1,033,229)

GB patent 1,197,164 relates to preparations for dental care and/or mouth care comprising proteolytic enzymes obtained from *Bacillus subtilis* as therapeutics. The use of said proteolytic enzymes improves the storage stability.

US patent 3,194,738 and FR patent 1,377,415 suggest the use of enzymes obtained from *Aspergillus oryzae* having proteolytic and amylolytic activity for toothpaste, chewing gum and mouth washes.

FR patent 1,448,385 concerns oral care products comprising proteolytic enzymes, such as animal derived carboxypeptidase, pepsin, trypsin, pancreatic chymotrypsin, cathepsin, and papain derived of vegetable origin, and other enzymes, such as lipases

US patent 4,364,926 (Yokogawa et al.) discloses a novel alkaline protease M_3 useful for preventing dental caries. The protease has a strong alkaline pH-optimum between 9 and 12.5, is not inhibited by a number of chemical compounds, and has glucosyltransferase (GTF) inhibitory activity.

US patent 4,986,981 (Glace et al.) concerns a pH neutral toothpaste having low abrasion. The toothpaste comprises papain in

an amount which is stated to effectively clean away plaque, mucin, and tartar.

Discussion of prior art

Oral care products comprising proteases may inflict bleedings in the mouth, and may damage or irritate the mouths mucus membrane. Even after rinsing the mouth with water, small amounts of protease constitutes such a risk.

Therefore, it is desirable to be capable of providing oral care compositions and products comprising proteases, which do not inflict any risk of causing damages in the mouth.

SUMMARY OF THE INVENTION

The object of the present invention is to overcome the above mentioned problem by providing oral care compositions and products, such as dentifrice compositions, comprising a protease as an active oral care component, which does not inflict any damages or irritations in the mouth cavity.

In the first aspect the invention relates to an oral care composition comprising a protease, which protease is substantially inactive in the natural environment prevailing in the mouth.

Further the invention relates to oral care products, such as toothpastes and mouth washes, comprising an oral care composition of the invention, or an essentially purified protease substantially inactive in the natural environment prevailing in the mouth, when the oral care composition or product is absent.

Another object of the invention is the use of proteases, substantially inactive in the natural environment prevailing in the mouth, in *e.g.* oral care compositions and products.

A final object of the invention is to provide a method for using said oral care products.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows the pH activity profile for protease I and protease II described in WO 95/02044.

Figure 2 shows the protein removing ability of protease I and protease II described in WO 95/02044 in comparison to said ability of Subtilisin Carlsberg, in a 3% H_2O_2 solution, pH 3.5, 4 hours, 25°C.

DETAILED DESCRIPTION OF THE INVENTION

The present invention solves the above mention problem related to oral care compositions and products comprising proteases.

The inventor has perceived that certain proteases advantageously can be used in oral care compositions and products. During use of oral care compositions and products a suitable environment is defined and created in the mouth, to facilitate the oral cleaning and/or treating process.

As one of the problems connected to prior art use of proteases in oral care compositions and products relate to the action of proteases after use, proteases, which are substantially inactive in the natural environment prevailing in the mouth, can be useful.

According to the invention parameters responsible for the protease inactivation may be any physical parameter, such as pH, temperature, humidity, ionic strength, etc. Especially contemplated is however the pH, as it is a relatively easy parameter to control.

Having in mind that the pH naturally prevailing in the mouth lies within the range from about 6 to 8, especially about pH 6.4 to 7.0 (pH of saliva), proteases being inactive within this pH-ranges do not inflict any damage or irritation in the mouth.

The advantageous known oral care action of proteases can be activated by adjusting the pH in the mouth to a suitable pH, where the protease has a suitable activity.

Therefore, if the protease is active in acidic environment the pH in the mouth is to be decreased. This can be done by adding suitable pH-decreasing agents to the oral care composition or product. However, if the pH of the oral care composition or product in it self is acidic, the pH adjustment is not necessary.

If the protease is active in alkaline environment the pH must be increased (if necessary), e.g. by adding pH-increasing agents.

When rinsing the mouth with water, after using a protease-containing oral care product, most of the protease will be removed from the mouth. However, even small amounts of remaining active protease may inflict damages in the mouth. By using an oral care product comprising a composition of the invention such damages can be avoided, as said composition comprises protease(s) which is(are) only active at the pH created by the oral care composition or product, and substantially inactive in the natural environment prevailing in the mouth. After rinsing the mouth with water the pH will, after a short period of time, return to a pH between about 6.4 to 7.0 (pH of salvia), which will inactivate the protease. Consequently, any amount of remaining protease will do no harm in the mouth.

Consequently the first aspect of the invention is to provide an oral care composition comprising a protease, wherein the protease is substantially inactive in the natural environment

prevailing in the mouth, when the oral care composition is absent.

It is to be understood that according to the invention the protease is active during use of the oral care composition, *e.g.* in connection with brushing the teeth or washing the mouth. After use any remains of active protease in the mouth will be inactivated, as the environment in the mouth will return to the natural stage, *i.e.* an environment primarily created by the saliva. Said return may optionally be speeded up by rinsing the mouth with water.

In a preferred embodiment said protease is substantially inactive in the natural pH-environment prevailing in the mouth, when the oral care composition is absent.

As mentioned above the pH of saliva ranges from about 6.4 to 7.0. However, the pH in the mouth may be transposed a little up or down *e.g.* in connection with eating and drinking. Therefore, proteases being substantially inactive in a close range of about pH 7, better within the pH-range from about 6.4 to 7.0, and even better within the pH-range from about pH 6 to 8, can be used as the protease component in an oral care composition or product of the invention. Proteases having a suitable relative activity (more than 70%) outside said range are preferred.

The temperature in the mouth is usually in the range of 20°C up to at most 37°C. Therefore, proteases having a high relative activity (more that 70%) within this temperature interval are preferred according to the invention. However, all proteases being active in the mouth within this temperature interval are contemplated according to the invention.

The above used terms "substantially inactive" define proteases having an activity so low that the proteases do not inflict any damages or irritations in the mouth, when the pH lies in a

close range of 7, preferably within the pH-range of saliva (about pH 6.4 to 7.0), more preferably in the pH-range from about 6 to 8.

More specifically this means that, within the above mentioned pH-ranges, the relative protease activity (determined from the protease activity described in the Methods and Materials section) must not exceed about 70%, and should preferably be below about 50%, more preferably below about 30%, especially below about 10%, even better below about 5%, such as below about 2%, determined on the basis of the proteolytic activity at the pH-optimum.

In an embodiment of the invention the protease(s) used in a composition of the invention is(are) derived from microorganisms, such as bacteria or fungal microorganisms.

Especially contemplated according to the invention is proteases derived from a strain of *Bacillus* sp., in particular a strain of *Bacillus subtilis*, or fungal strains of a *Rhizopus* sp., in particular a strain of *R. niveus*, or a strain of a *Schytalidium* sp., or a strain of a *Sulpholobus* sp., or a strain of a *Thermoplasma* sp., or a strain of a *Aspergillus* sp., in particular a strain of *A. aculeatus*, or *A. niger*, or *A. awamori*, or *A. oryzae*, or a strain of a *Trichoderma* sp., in particular a strain of *T. harzianum*, or *T. reesie*, or a strain of a *Fusarium* sp., in particular a strain of *F. oxysporum*, or a strain of a *Humicola* sp..

According to the invention the protease may be either an acidic or alkaline protease, within the group of proteases (i.e. enzymes classified under the Enzyme Classification number E.C. 3.4 in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB)), which is substantially inactive in the natural environment prevailing in the mouth, when the oral care composition or product is absent.

Furthermore, also genetically modified proteases designed for the purpose of the invention are contemplated. Said modified proteases can be selected by screening for protease variants having a suitable pH-profile. Said variants may be provided by site directed or cassette mutagenesis as described in e.g. EP 130.756 (Genencor) or EP 479.870 (Novo Nordisk A/S); or by random mutagenesis by using methods well established in the art.

In a specific embodiment of the invention the protease has a protease activity pH-optimum at about pH 5.

Specific examples of such proteases are the acidic proteases, exemplified with protease I and protease II, respectively, described in WO 95/02044 (Novo Nordisk A/S).

These acidic proteases are substantially inactive in the natural environment prevailing in the mouth, including the natural pH-environment prevailing in the mouth.

Protease I and protease II are surprisingly better than expected, as the proteases are substantially inactive in the natural environment in the mouth. Protease I and protease II are substantially inactive within the pH-range from about pH 6 to 8, and have relative protease activities being less than 50% within the pH-range from about 6.4 to 7.0. Within a close range of about pH 7, the relative protease activities are insignificant for protease I, and substantilally reduced for protease II (see figure 1 and example 1). Further, Protease I and protease II have good protein removing activities in the presence of as high as up to 3% H_2O_2 (see figure 2 and example 2). This is advantageous as H_2O_2 is used in oral care products, such as dentifrices, as whitening/bleaching agent.

A protease is, in the context of the invention, regarded as having "a good protein removing activity" if it is capable of remove significantly more protein from a surface than

Subtilisin Carlsberg (available from Novo Nordisk A/S as Alcalase®) at the same conditions in 3% H_2O_2.

The amount of protease needed in an oral care composition or product of the invention depends on the particular compound employed, but ranges generally from 0.0001% to 20%, preferably from about 0.001% to about 10%, and most preferably from about 0.01% to about 5% by weight of the final product.

In the preparation of an oral care composition or product, the protease may be added as an essentially purified mono-component enzyme preparation, such as an enzyme preparation containing one of the proteases described in WO 95/02044 (Novo Nordisk A/S), but may also be added as an enzyme preparation exhibiting at least one other activity. Contemplated activities include the activity of a reducing enzyme, such as peroxidase, and further mutanase, dextranase, lipase, amylase, glucose oxidase activity, and anti-microbial polypeptides, enriched with a protease which is substantially inactive in the natural environment prevailing in the mouth of humans, and animals, such as dogs and cats.

In a preferred embodiment of the invention the oral care composition comprises protease, dextranase and/or mutanase activity.

An oral care composition according to the invention may advantageously be used for producing an oral care product having any suitable physical form (*i.e.* powder, paste, gel, liquid, ointment, tablet etc.). Said "oral care product" is defined as a product which effectively can be used for maintaining and/or improving oral hygiene in the mouth of humans and animals, and/or preventing or treating dental diseases.

Examples of such oral care products include toothpaste, dental cream, gel or tooth powder, odontic, mouth washes, pre- or post brushing rinse formulations, chewing gum, lozenges, and candy.

Toothpastes and tooth gels typically include abrasive polishing materials, foaming agents, flavouring agents, humectants, binders, thickeners, sweetening agents, whitening/bleaching/stain removing agents, and water.

Mouth washes, including plaque removing liquids, typically comprise a water/alcohol solution, flavour, humectant, sweetener, foaming agent, and colorant.

According to the invention said abrasive polishing material includes alumina and hydrates thereof, such as alpha alumina trihydrate, magnesium trisilicate, magnesium carbonate, kaolin, aluminosilicates, such as calcined aluminum silicate and aluminum silicate, calcium carbonate, zirconium silicate, and also powdered plastics, such as polyvinyl chloride, polyamides, polymethyl methacrylate, polystyrene, phenol-formaldehyde resins, melamine-formaldehyde resins, urea-formaldehyde resins, epoxy resins, powdered polyethylene, silica xerogels, hydrogels and aerogels and the like. Also suitable as abrasive agents are calcium pyrophosphate, water-insoluble alkali metaphosphates, dicalcium phosphate and/or its dihydrate, dicalcium orthophosphate, tricalcium phosphate, particulate hydroxyapatite and the like. It is also possible to employ mixtures of these substances.

Dependent on the oral care product the abrasive product may be present in from 0 to 70% by weight, preferably from 1% to 70%. For toothpastes the abrasive material content typically lies in the range of from 10% to 70% by weight of the final toothpaste product.

Humectants are employed to prevent loss of water from e.g. toothpastes. Suitable humectants for use in oral care products according to the invention include the following compounds and mixtures thereof: glycerol, polyol, sorbitol, polyethylene glycols (PEG), propylene glycol, 1,3-propanediol, 1,4-butanediol, hydrogenated partially hydrolysed polysaccharides

and the like. Humectants are in general present in from 0% to 80%, preferably 5 to 70% by weight in toothpaste.

Silica, starch, tragacanth gum, xanthan gum, extracts of Irish moss, alginates, pectin, cellulose derivatives, such as hydroxyethyl cellulose, sodium carboxymethyl cellulose and hydroxypropyl cellulose, polyacrylic acid and its salts, polyvinylpyrrolidone, can be mentioned as examples of suitable thickeners and binders, which helps stabilizing the dentifrice product. Thickeners may be present in toothpaste creams and gels in an amount of from 0.1 to 20% by weight, and binders to the extent of from 0.01 to 10% by weight of the final product.

As foaming agent soap, anionic, cationic, non-ionic, amphoteric and/or zwitterionic surfactants can be used. These may be present at levels of from 0% to 15%, preferably from 0.1 to 13%, more preferably from 0.25 to 10% by weight of the final product.

Surfactants are only suitable to the extent that they do not exert an inactivation effect on the present protease. Surfactants include fatty alcohol sulphates, salts of sulphonated mono-glycerides or fatty acids having 10 to 20 carbon atoms, fatty acid-albumen condensation products, salts of fatty acids amides and taurines and/or salts of fatty acid esters of isethionic acid.

Suitable sweeteners include saccharin.

Flavours, such as spearmint, are usually present in low amounts, such as from 0.01% to about 5% by weight, especially from 0.1% to 5%.

Whitening/bleaching agents include H_2O_2 and may be added in amounts less that 5%, preferably from 0.25 to 4%, calculated on the basis of the weight of the final product.

Water is usually added in an amount giving *e.g.* toothpaste a flowable form.

Further water-soluble anti-bacterial agents, such as chlorhexidine digluconate, hexetidine, alexidine, quaternary ammonium anti-bacterial compounds and water-soluble sources of certain metal ions such as zinc, copper, silver and stannous (*e.g.* zinc, copper and stannous chloride, and silver nitrate) may also be included.

Also contemplated according to the invention is the addition of anti-calculus agents, anti-plaque agents, compounds which can be used as fluoride source, dyes/colorants, preservatives, vitamins, pH-adjusting agents, anti-caries agents, desensitizing agents etc.

A toothpaste produced from an oral care composition of the invention (in weight % of the final toothpaste composition) may *e.g.* comprise the following ingredients:

Abrasive material	10 to 70%
Humectant	0 to 80%
Thickener	0.1 to 20%
Binder	0.01 to 10%
Sweetener	0.1% to 5%
Foaming agent	0 to 15%
Whitener	0 to 5%
Protease (according to the invention)	0.0001% to 20%
Other enzymes	0 to 20%

Another object of the invention relates to the use of proteases, which are substantially inactive in the natural environment prevailing in the mouth, in *e.g.* oral care compositions and products.

In a preferred embodiment the proteases are substantially inactive in the natural pH-environment prevailing in the mouth, within a close range of about pH 7, preferably within the pH-range from about 6.4 to 7.0, and even better within the pH-range from about 6 to 8.

Finally the invention relates to a method for using an oral care product of the invention, wherein

a) the oral care product is placed in the mouth,
b) contacted with the teeth and/or gums for a period of time,
c) removed from the mouth, and
d) optionally rinsed with a liquid.

If the oral care product to be used is in solid to flowable form a tooth brush or the like may advantageously be used for contacting the oral care product with the teeth and/or gums. In the case of a liquid oral care product the contact may take place by rinsing the mouth.

The time period of contact in step b) is optional. However, contacting the oral care product with the teeth and/or gums for between about 1 to 5 minutes will normally be sufficient for obtaining the desired result.

After use, the oral care product may be removed from the mouth in any suitable way, *e.g.* by spitting it out. Optionally the mouth may be rinsed with a liquid, such as tap water. This will secure that the environment, *e.g.* the pH-environment, in the mouth returns to the natural stage within a short period of time.

MATERIALS AND METHODS

Materials

Protease I is described in WO 95/02044

Protease II is described in WO 95/02044

Subtilisin Carlsberg is available from Novo Nordisk A/S as Alcalase®.

Methods

Proteolytic activity

1 haemoglobin protease unit (hpu) is defined as the amount of enzyme liberating 1 millimole of primary amino groups (determined by comparison with a serine standard) per minute under standard conditions as described below:

A 2% (w/v) solution of haemoglobin (bovine, supplied by Sigma) is prepared with the Universal Buffer described by Britton and Robinson, J. Chem. Soc., 1931, p. 1451), adjusted to a pH of 5.5. 2 ml of the substrate solution are pre-incubated in a water bath for 10 min. at 25°C. 1 ml of an enzyme solution containing b g/ml of the enzyme preparation, corresponding to about 0.2-0.3 hpu/ml of the Universal Buffer (pH 5.5) is added. After 30 min. of incubation at 25°C, the reaction is terminated by the addition of a quenching agent (5 ml of a solution containing 17.9 g of trichloroacetic acid, 29.9 g of sodium acetate and 19.8 g of acetic acid made up to 500 ml with deionized water). A blank is prepared in the same way as the test solution with the exception that the quenching agent is added prior to the enzyme solution. The reaction mixtures are kept for 20 min. in a water bath after which they are filtered through Whatman 42 paper filters.

Primary amino groups are determined by their colour development with o-phthaldialdehyde (OPA), as follows: 7.62 g of disodium tetraborate decahydrate and 2.0 g of sodium dodecylsulfate are dissolved in 150 ml of water. 160 mg of OPA dissolved in 4 ml of methanol were then added together with 400 µl of ß-mercaptoethanol after which the solution is made up to 200 ml with water. To 3 ml of the OPA reagent are added 400 µl of the filtrates obtained above, with mixing. The optical density (OD) at 340 nm is measured after about 5 min. The OPA test is also performed with a serine standard containing 10 mg of serine in

100 ml of Universal Buffer (pH 5.5). The buffer alone is used as a blank. The protease activity is calculated from the OD measurements by means of the following formula:

$$\text{hpu/ml enzyme solution:} \quad \frac{(OD_t - OD_b) \times C_{ser} \times Q}{(OD_{ser} - OD_B) \times MW_{ser} \times t_i}$$

hpu/g of enzyme preparation = hpu/ml: b

wherein OD_t, OD_b, OD_{ser} and OD_B is the optical density of the test solution, blank, serine standard and buffer, respectively, C_{ser} is the concentration of serine (mg/ml) in the standard (in this case 0.1 mg/ml), and MW_{ser} is the molecular weight of serine (105.09). Q is the dilution factor for the enzyme solution (in this case 8) and t_i is the incubation time in minutes (in this case 30 minutes).

EXAMPLES

Example 1

Activity profile

To illustrate the existence of suitable proteases for oral care compositions and products of the invention the pH activity profiles in relative percents of protease I and protease II described in WO 95/02044 were determined.

As can be seen from figure 1 the relative protease activity has disappeared at pH 7 for protease I, and has been substantially reduced for protease II.

Example 2

Protease activity in 3% H_2O_2

To illustrate the useful effect of suitable proteases in oral care products, the ability to remove protein from a surface in a 3% H_2O_2 solution, 25°C, 4 hours, pH 3.5, was tested. The ability of proteases I and protease II described in WO 95/02044 was compared with Subtilisin Carlsberg.

As can be seen from figure 2 both protease I and protease II are far more effective for removing protein than Subtilisin Carlsberg. This makes both proteases highly useful for oral care compositions and products containing H_2O_2.

As will be apparent to those skilled in the art in the light of the foregoing disclosure, many alterations and modifications are possible in the practice of this invention without departing from the spirit or scope thereof. Accordingly, the scope of the invention is to be construed in accordance with the substance defined by the following claims.

PATENT CLAIMS

1. An oral care composition comprising a protease, characterized in that the protease is substantially inactive in the natural environment prevailing in the mouth, when the oral care composition is absent.

2. The oral care composition according to claim 1, wherein the protease is substantially inactive in the natural pH-environment prevailing in the mouth, when the oral care composition is absent.

3. The oral care composition according to any of claims 1 and 2, wherein the protease is substantially inactive in the close range of about 7, preferably with the range of about pH 6.4 to 7.0, even more preferred between about pH 6 to 8.

4. The oral care composition according to claims 3, wherein the protease is active at temperatures between 20°C and 37°C.

5. The oral care composition according to claims 3 and 4, wherein the proteolytic activity is below 70%, preferably below 50%, more preferably below 30%, better below 10%, even better below 5%, such as below 2%, in comparison to the proteolytic activity at the pH-optimum.

6. The oral care composition according to any of claims 1 to 5, wherein the protease is derived from a microorganism.

7. The oral care composition according to claim 6, wherein the protease is derived from a strain of *Bacillus* sp., in particular a strain of *Bacillus subtilis*, or fungal strains of a *Rhizopus* sp., in particular a strain of *R. niveus*, or a strain of a *Schytalidium* sp., or a strain of a *Sulpholobus* sp., or a strain of a *Thermoplasma* sp., or a strain of a *Aspergillus* sp., in particular a strain of *A. aculeatus*, or *A. niger*, or *A. awamori*, or *A. oryzae*, or a strain of a *Trichoderma* sp., in

particular a strain of *T. harzianum*, or *T. reesie*, or a strain of a *Fusarium* sp., in particular a strain of *F. oxysporum*, or a strain of a *Humicola* sp..

8. The oral care composition according to any of claims 1 to 7, wherein the protease is an acidic protease.

9. The oral care composition according to claim 8, wherein the acidic protease is a genetically modified protease.

10. The oral care composition according to any of claims 1 to 9, wherein the protease has a good protein removing activity in the presence of H_2O_2.

11. The oral care composition according to any of claims 1 to 8, and 10 wherein the protease is protease I described in WO 95/02044.

12. The oral care composition according to any of claims 1 to 8, and 10, wherein the protease is protease II described in WO 95/02044.

13. The oral care composition according to any of claims 1 to 7, wherein the protease is an alkaline protease.

14. The oral care composition according to claim 13, wherein the alkaline protease is a genetically modified protease.

15. The oral care composition according to claims 13 and 14, wherein the protease has a good protein removing activity in the presents of H_2O_2.

16. The oral care composition according to any of claims 1 to 15, comprising at least one other activity selected from the group including a reducing enzyme, such as peroxidase, and amylase, dextranase, mutanase, lipase, glucose oxidase, and anti-microbial polypeptides, or a mixture thereof.

17. The oral care composition according to claim 16, comprising dextranase or mutanase, or a mixture thereof.

18. An oral care product, comprising an oral care composition according to any of claims 1 to 17, or a protease being substantially inactive in natural environment prevailing in the mouth, when the oral care product is absent.

19. An oral care product according to claim 18, wherein said protease is substantially inactive the natural pH-environment prevailing in the mouth, when the oral care product is absent.

20. An oral care product according to claims 18 and 19, being a dentifrice.

21. The oral care product according to claim 20, wherein the dentifrice is a toothpaste or tooth powder.

22. The oral care product according to claim 20, wherein the dentifrice is a mouth wash.

23. The oral care product according to any of claims 18 to 22, comprising an anti-plaque compound or composition.

24. The oral care product according to any of claims 18 to 23, comprising an anti-tartar compound or composition.

25. The oral care product according to any of claims 18 to 24, comprising a bleaching or whitening agent.

26. The oral care product according to claim 25, comprising H_2O_2.

27. The oral care product according to any of claims 18 to 26, comprising at least one other activity selected from the group including a reducing enzyme, such as peroxidase, amylase,

dextranase, mutanase, lipase, glucose oxidase or anti-microbial activity, or a mixture thereof.

28. The oral care product according to claim 27, comprising dextranase or mutanase, or a mixture thereof.

29. Use of a protease, which is substantially inactive in the natural environment prevailing in the mouth.

30. The use according to claim 29, wherein the protease is substantially inactive in the natural pH-environment prevailing in the mouth.

31. The use according to claim 29 and 30, in oral care compositions and/or products.

32. A method for using oral care products according to any of claims 18 to 28, wherein
a) the oral care product is placed in the mouth,
b) contacted to the teeth and/or gums for a period of time,
c) removed from the mouth, and
d) optionally rinsed with a liquid.

29. The method according to claim 29, wherein a tooth brush is used for contacting the oral care product with the teeth and/or gums.

30. The method according to claims 29 and 30, wherein the oral care product is contacted with the teeth and/or gums by rinsing.

31. The method according to any of claims 29 to 31, wherein the rinsing liquid in step d) is water.

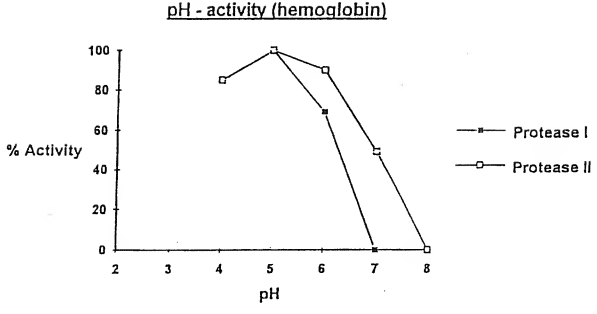
pH - activity (hemoglobin)
% Activity
100
80
60
40
20
0
2
3
4
5
6
7
8
pH
Protease I
Protease II

Fig. 1

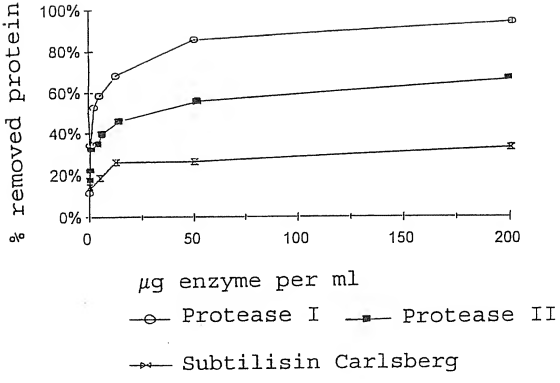
% removed protein
100%
80%
60%
40%
20%
0%
0
50
100
150
200
µg enzyme per ml
Protease I
Protease II
Subtilisin Carlsberg

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00126

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 7/28
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, BIOSIS, CLAIMS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0258186 A2 (WARNER-LAMBERT COMPANY), 2 March 1988 (02.03.88), page 2, line 47 - line 61; page 3, line 6 - line 13; page 3, line 27 - line 28, column 4, line 1 - line 5, claim 1	1-31
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X	Dialog Information Services, File 347, JPO & JAPIO, Dialog accession no. 03275316, Kao Corp: "Composition for oral cavity application", & Section: C, Section no. 790, Vol 14, No. 571, Pg. 129, December 19, 1990, abstract	1-31
	--	
A	WO 9502044 A1 (NOVO NORDISK A/S), 19 January 1995 (19.01.95), claims 14,16-20	1-31
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 July 1996	11 -07- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Authorized officer Dagmar Järvman Telephone No. + 46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00126

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4364926 A (K. YOKOGAWA ET AL), 21 December 1982 (21.12.82)	1-31

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INTERNATIONAL SEARCH REPORT

Information on patent family members

01/04/96

International application No.

PCT/DK 96/00126

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0258186	02/03/88	SE-T3- 0258186	
		AU-B,B- 581126	09/02/89
		AU-A- 7608487	03/03/88
		JP-A- 63101313	06/05/88
		ZA-A- 8705318	25/01/88
WO-A1- 9502044	19/01/95	NONE	
US-A- 4364926	21/12/82	DE-A,A- 3102216	04/03/82
		FR-A,B- 2482131	13/11/81
		GB-A,B- 2069501	26/08/81
		JP-C- 1429373	09/03/88
		JP-A- 56106593	24/08/81
		JP-B- 62036671	07/08/87